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Some Studies on the Chemistry and the Physiology of the Parathyroid Hormone

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SOME STUDIES ON THE CHEMISTRY AND THE PHYSIOLOGY OF THE PARATHYROID HORMONE

by

George Henry Smullen

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ACKNOWLEDGMENT

I wish to acknowledge my indebtedness to Dr. W. R. Tweedy for the advice and assistance he has so kindly and consistently given me, and for the opportunity of advancing myself in this particular branch of scientific research.

I also wish to thank Dr. W. C. Austin for the many courtesies he has accorded me throughout the year.

VITA

I was born in Johnstown, New York on July 3, 1911. I attended the Johnstown Public Schools and the Chicago Public Schools; and was graduated from the Mulligan elementary school in the spring of 1926 and from the Robert A. Waller High School in the summer of 1929. Following two years of pre-medical work (1930-1932) at Loyola University College of Arts and Sciences, I entered Loyola University School of Medicine in the fall of 1932. My Bachelor of Science in Medicine was received in 1934. I was teaching fellow in the Department of Physiological Chemistry during the year of 1934-1935.

INTRODUCTION

In 1926 Tweedy (1) developed a method for the extraction of a serum calcium-raising principle from bovine parathyroid glands.

In subsequent years Tweedy and co-workers have attempted to further purify and characterize the hormone. J. J. Smullen (2) succeeded in developing a method for further purification. The presence of certain chemical groups in the hormone molecule is indicated by the findings of M. Torigoe (3), W. P. Bell (4), and C. Vicens-Rios (5).

The experimental work discussed in this thesis has been carried out for the purpose of obtaining additional information of the chemistry and the physiology of the parathyroid hormone.

The chemical studies have included a new method of purification of the hormone; nitrogen partition of the hormone; acid hydrolysis under atmospheric conditions and under increased pressure, in the presence of air and in the presence of a single inert gas; adsorption of the hormone on activated charcoal and on Lloyd's reagent; and the attempted reduction of the hormone by sodium in liquid ammonia.

The physiological studies attempt to show absorption of the hormone from the G. I. tract, and the effect of administration of the hormone intravenously.

EXPERIMENTAL

PART I STUDIES ON THE CHEMISTRY OF THE PARATHYROID HORMONE

EXTRACTION OF THE HORMONE

The hormone was extracted during the past summer by the method of Tweedy.

METHOD OF PURIFICATION

A number of preliminary experiments were carried out in which varying amounts of unpurified parathyroid hormone preparation were dissolved in 92.5% to 98.4% acetic acid and then precipitated them with five volumes of anhydrous acetone. The precipitates were washed with acetone until the acid reaction to blue litmus paper disappeared. Finally, the precipitates were dried with anhydrous ether and then over calcium chloride in a dessicator in vacuo.

On the basis of these preliminary tests the following procedure was adopted:

63.61 grams of unpurified hormone preparation were dissolved as completely as possible in 1280 cc. of 93.3% acetic acid. The residue was dissolved in 100 cc. of additional 93.3% acetic acid and mixed with the above solution. The acetic acid solution of the hormone was divided into three portions.

To portion #1 (500 cc.) were added 2500 cc. of fresh

acetone. To portion #2 (500 cc.) were added 2500 cc. of drain acetone from #1 plus 500 cc. of fresh acetone. To portion #3 (380 cc.) were added 2000 cc. of drain acetone from #2 plus 2 volumes (approximately 800 cc.) of fresh acetone.

The precipitates were allowed to settle and the supernatant liquid was siphoned off and saved for further extraction.

The precipitates were transferred to 50 cc. centrifuge tubes and washed several (10-12) times with anhydrous acetone until the washings gave no acid reaction to blue litmus paper. Finally the precipitates were washed with anhydrous ether, dried at room temperature, pulverized into a fine powder, placed in a dessicator and dried over $CaCl_2$ in vacuo. The recovery was as follows:

Weight of Initial Material.....63.6101 gms.
Weight of Purified Material.....39.4717 gms.
Percentage Recovered.....62.05 %

The hormone material which had been left in the acetone-acetic acid solution was recovered by evaporating the solution under reduced pressure. The mother liquor consisted of a dark reddish brown liquid, mostly acetic acid. It was found that when three volumes of anhydrous ether were added to one volume of this liquid a light brown precipitate would form. The precipitates were washed with anhydrous ether until no acid reaction was shown to blue litmus paper.

The material contained very little, if any, activity as was shown by the following potency tests:

Dog	Sex	Wt. kg.	Dose	Init. Serum Ca.	Final Ca.	Ca. Incr't.
C6	M	24.3	72.9 mg.	11.24	11.86	0.62

METHOD OF BIOLOGICAL ASSAY

Twenty-seven dogs were used for the standardization of the purified hormone. About five o'clock P. M. the blood sample was taken before injecting the hormone material subcutaneously and the second sample--approximately 15 hours later.

Normal dogs were injected subcutaneously with the same dose per kilogram of body weight. Blood serum calcium analyses were made according to the Tweedy-Koch (6) modification of the Kramer-Tisdall method. Duplicate analyses were made for each animal. According to our assay 3.5 mgs. of the parathyroid hormone preparation were equivalent to 100 units since according to Collip's (7) method of standardization 100 units of parathyroid hormone constitute an amount sufficient to increase the blood serum calcium of a dog weighing 20 kilos 5 mgs. per cent over a period of 15 hours. (See Table I)

NITROGEN PARTITION OFTHE PARATHYROID HORMONE

The total nitrogen content of the parathyroid hormone

TABLE I

BLOOD SERUM CALCIUM INCREMENT PRODUCED BY ADMINISTRATION OF PARATHYROID HORMONE

No.	Dog	Wt. kg.	Dose 3.5 mg/kg	Initial Calcium mg.%	Final Calcium mg.%	Calcium Increment mg.%
1	B6	26.3	78.9	10.84	14.62	3.78
2	B1	25.0	75.0	10.96	14.85	3.89
3	B3	24.5	74.0	11.28	15.07	3.79
4	D6	24.5	73.5	10.96	13.76	2.80
5	D2	21.8	65.4	12.07	14.18	2.11
6	B2	20.9	63.0	10.75	14.29	3.54
7	B4	20.4	61.2	10.50	14.58	4.08
8	C5	19.1	57.3	11.34	17.25	5.91
9	C4	19.0	57.0	11.74	15.81	4.07
10	B5	17.3	51.9	10.88	13.15	2.27
11	D3	16.3	48.9	9.57	16.20	6.63
12	B7	16.0	48.0	11.28	15.96	4.68
13	B8	12.5	37.5	11.07	15.54	4.47
14	D8	11.3	33.9	10.77	13.38	2.61
15	D7	9.7	29.1	11.00	13.90	2.90
16	C1	9.0	27.0	9.99	12.15	2.16
17	D5	9.0	27.0	11.53	14.37	2.84
18	C2	6.8	20.4	11.12	14.71	3.59

TABLE I

BLOOD SERUM CALCIUM INCREMENT PRODUCED BY ADMINISTRATION OF PARATHYROID HORMONE

No.	Dog	Wt. kg.	Dose 3.5 mg/kg	Initial Calcium mg.%	Final Calcium mg.%	Calcium Increment mg.%
19	E3	15.4	53.90	11.81	20.07	8.26
20	E5	14.5	50.75	11.24	15.14	3.90
21	F1	14.5	50.75	11.09	13.90	2.81
22	E4	14.1	49.35	11.47	16.74	5.27
23	E6	13.8	48.30	11.40	14.22	2.82
24	F3	10.0	35.00	11.62	13.36	1.74
25	F2	9.0	31.50	10.87	11.08	0.11
26	F5	7.7	26.95	10.80	13.87	3.17
27	F4	6.8	21.80	11.62	18.04	3.09

preparation was determined and checked by several analyses, using the Koch- McMeekin micro kjeldahl method (8). A value of 14.74% was found.

The nitrogen partition found by the Thimann procedure (9) was as follows:

Acid amide nitrogen.....4.39%

Humin nitrogen.....0.92%

Dibasic nitrogen.....21.13%

Non-basic nitrogen.....71.53%

The available peptide nitrogen was found to be 72.5% of the total nitrogen content or 10.66 mg. of N per 100 mg. of hormone. It was determined as follows: 50 mg. of hormone were dissolved in 10 cc. of 20% HCl and placed in a flask which was connected to a condenser. The flask was placed on a sand bath over an electric plate. After 25 hours of continuous heating the solution was evaporated in vacuo over a hot water bath. When the material was concentrated to about one cc. the volume was increased to 10 cc. by the addition of distilled water. The evaporation and the addition of water were repeated. The free amino acid nitrogen content was then determined by the Van Slyke method (10).

ACID HYDROLYSIS OF PARATHYROID HORMONE

Collip (11) stated that the parathyroid hormone is inactivated by boiling for 1 hour in 10% HCl. He did not state, however, how much destruction or hydrolysis of the hormone

had taken place. By a measurement of the free amino acid nitrogen it was found that when the hormone is boiled for 1 hour in 10% HCl the free amino acid nitrogen increases from an initial value of 6.51% to 33.21% of the total nitrogen. Hydrolysis does not have to go this far to produce inactivation of the hormone, for when the free amino acid nitrogen reaches a value of 12.5% of the total nitrogen content, potency is destroyed.

When the parathyroid hormone preparation is treated with concentrated HCl (35%) for 1 hour at room temperature (23°C.) potency is retained.

Dog	Wt. kg.	Dose	Init. Ca	Final Ca	Ca Increment
C 8	24	72 mg.	11.55	14.45	2.80
			11.65	14.60	2.95
			11.60	14.52	2.87

Even when the the temperature is raised to 35°C. by means of a water bath and the hormone treated with concentrated HCl (35%) for 1 hour, potency is still retained.

Dog	Wt. kg.	Dose	Init. Ca	Final Ca	Ca Increment
C 9	15.5	37.5 mg.	11.44	13.55	2.11
			11.44	13.75	2.31
			11.44	13.65	2.21

A series of acid hydrolysis experiments were carried out in an attempt to correlate the increase or loss of po-

tency ofthe hormone with the amount of hydrolysis that had taken place (1) over a given period, (2) in a given concentration of acid, and (3) at a given temperature. (1), (2), or (3) were varied in different experiments in order to arrive at a definite conclusion as to the effect of each variable.

The amount of hydrolysis was measured by determining the free amino acid nitrogen content of the hormone both before and after hydrolysis by means of the Van Slyke apparatus. The values for the free amino acid nitrogen were converted to their equivalents in mg. of nitrogen and compared with the total nitrogen contentand the available peptide nitrogen.

The description of a typical experiment will suffice for an explanation of the method used in the study of acid hydrolyses: 300 mg. of parathyroid hormone preparation were dissolved in 10 cc. of .05 N. HCl. $2\frac{1}{2}$ cc. were removed for the initial free amino acid nitrogen determination by the Van Slyke method. The remainder ($7\frac{1}{2}$ cc.) were placed in a flask which was connected, by ground glass connections, to a reflux condenser. The flask was placed over a boiling water bath for x hours. After the allotted time of digestion the contents were removed, quantitatively, and made up to a volume of exactly 10 cc. Another $2\frac{1}{2}$ cc. portion was removed for the determination of the final free amino acid nitrogen

value. The remaining 7½ cc. were used to determine the potency of the hydrolyzed hormone. This was done by taking a blood sample of a normal dog in the evening, determining the calcium content of the serum, injecting this dog subcutaneously with 3.5 mg. of hormone per kilo of body weight, and on the following morning (15 hours after injection) determining the calcium content of another blood serum sample.

The free amino acid nitrogen content of the hormone preparation is 4.53% of the total nitrogen or 6.51% of the determined peptide nitrogen. When the hormone preparation is heated for 5 hours in 0.05 N. HCl on a boiling water bath the free amino acid nitrogen content is increased to 7.35% of the total nitrogen or 10.13% of the available peptide nitrogen with no loss of potency (Table 2). With 10 hours of heating in 0.05 N. HCl the free amino acid nitrogen is increased to 8.55% of the total nitrogen or 11.78% of the available peptide nitrogen. Although the free amino acid nitrogen value is almost doubled there is no apparent loss of potency (Table 2).

When the free amino acid nitrogen is increased to 9.11% of the total nitrogen by boiling for 20 hours in 0.05 N. HCl there is still a very slight amount of activity left in the hormone preparation (Table 2).

Even when the free amino acid nitrogen content of the hormone is increased to 10.85% of the total nitrogen or 14.98% of the available peptide nitrogen the hormone pos-

sesses some potency for when a one and one-half standard dose is injected subcutaneously into a dog there is a decided rise in the blood serum calcium (Table 2).

Table 3 illustrates the relation of the potency retained by the hormone when it is hydrolyzed in a 0.1 N HCl for various periods of time. The more gradual hydrolysis obtained with 0.05 N. HCl enables one to follow the loss of potency more easily.

The data for Table 2 are shown in condensed form in Chart I. The abscissa on the left represent the per cent of free amino acid nitrogen; on the right, the per cent of potency retained; the ordinates, the duration of hydrolysis. The potency curve is shown as the dotted line. The solid line represents the increase of free amino acid nitrogen.

TABLE II

.05 N HCl

Time of Hydrolysis Hrs.	Before Hydrolysis Init. NH_2-N / Total N %	Before Hydrolysis Init. NH_2-N / Available Peptide N %	After Hydrolysis Final NH_2-N / Total N %	After Hydrolysis Final NH_2-N / Available Peptide N %	Ca. Increment mg. %
5	4.53	6.51	7.35	10.13	2.12 7.81
10	4.53	6.51	8.55	11.78	4.59 3.44 3.67 4.14 3.96 0.34
20	4.53	6.51	9.11	12.57	0.86
25	4.53	6.51	10.85	14.98	0.34 0.11 *2.85

*-Dose and half.

Each value for a calcium increment was obatined by a separate potency determination.

The amino N is expressed asper cent of the total N and the peptide N.

TABLE III

0.1 N HCl

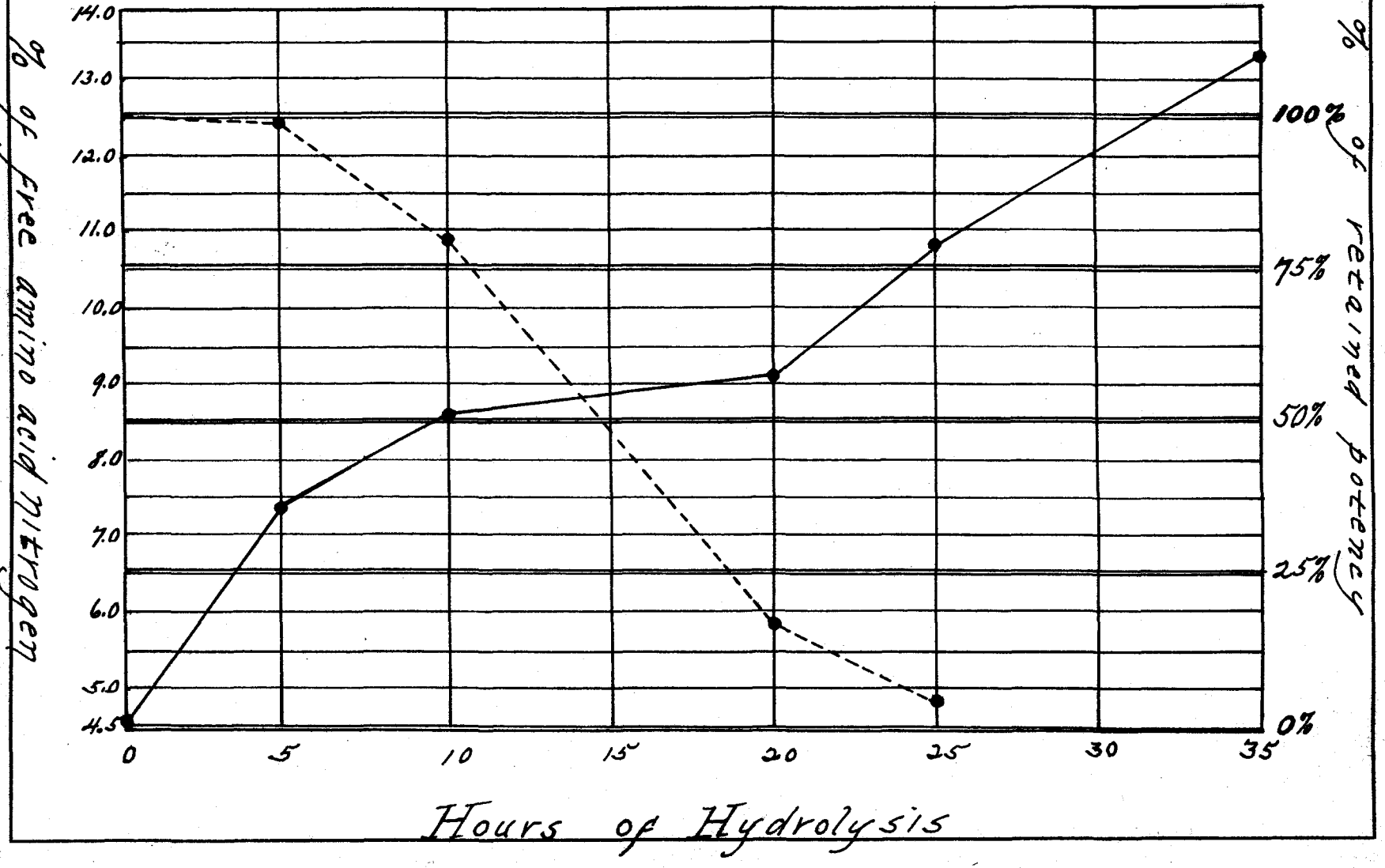
Time of Hydrolysis Hrs.	Before Hydrolysis Init. NH_2-N Total N %	Init. NH_2-N Available Peptide N %	After Hydrolysis Final NH_2-N Total N %	Final NH_2-N Available Peptide N %	Ca. Increment mg. %
1	4.53	6.51	----	----	2.71
p.33					
2	4.53	6.51	8.76		1.69
p.42					*4.59
	Filtrate		Filtrate		
19	4.12	6.51	8.63		1.95
	Ppt.				
p.66	0.49	6.51			@1.50

*- Unilateral nephrectomy.

@ - Dose and half.

CHART I

THE EFFECT OF BOILING 0.05 N. HCl ON THE POTENCY AND FREE AMINO ACID N. VALUES OF THE HORMONE PREPARATION



There are several possible explanations for the loss of potency when the hormone preparation is heated with a mineral acid. The most plausible explanations are:

(1) An essential linkage in the hormone molecule is hydrolyzed.

(2) The hormone preparation may be a mixture of proteins, proteoses, etc.; and the active principle may be a polypeptide in this mixture. If so, then during hydrolysis this polypeptide may be hydrolyzed to amino acids, thus destroying its potency.

(3) Atmospheric oxidation may play a role in the destruction of the active group or groups.

Our observations favor (1) or (2), but rule out (3) as the cause of destruction of potency.

If inactivation were due to atmospheric oxygen, then by protecting the hormone from the air during the heat treatment in acid, oxidation could be ruled out as the method of destruction of activity of the hormone. Such was the case.

Four methods were used in this study:

(1) Hydrolysis of the hormone with acid under a layer of paraffin oil which largely excluded atmospheric oxygen.

(2) Hydrolysis of the hormone under a layer of paraffin oil with a piece of zinc in the acid--hormone solution to react with acid to liberate hydrogen gas which served to drive off

any oxygen that might be present.

(3) Passage of a stream of nitrogen gas or oxygen-free carbon dioxide gas through the hormone preparation being hydrolyzed thus preventing atmospheric oxygen from coming in contact with the solution.

(4) Hydrolysis of the hormone--acid preparation in a steam pressure cooker after all the air had been rapidly replaced by water vapor.

One typical experiment from each of the above groups of experiments will suffice to illustrate the results obtained.

(1) Hydrolysis of the Hormone in Acid Under a Layer of Paraffin Oil

This type of experiment was carried out as follows:

120 mg. of hormone were dissolved in 4 cc. of 10% HCl in a centrifuge tube. A layer of paraffin oil was placed on the surface of the centrifuge tubes. To tube #1 were added 2 cc. of 5.8% HCl, a small piece of zinc, and a layer of paraffin oil. Zinc was added to liberate hydrogen gas and thus aerate out any oxygen present. Thus, if activity were lost, it would not be due to oxidation but to some other action, presumably splitting of the peptide linkages.

To tube #2 were added 2 cc. of 5.8% HCl to act as control. Both tubes were placed in a water bath which was gradually heated to boiling and kept at maximum temperature for

20 minutes. At the end of the heating period the contents of both tubes were neutralized and tested for potency.

Results:

Dog	Sex	Wt. kg.	Material	Init. Ca.	Final Ca.	Ca. Increment
I2	F	15.9	Tube #1	10.66	12.04	1.38
I3	F	20.4	Tube #2	10.89	13.53	2.64

According to the above results, there was a greater loss of potency in the tube containing hormone, zinc, and a layer of paraffin oil, showing that some action other than oxidation had reduced its potency.

(3) Hydrolysis of the Hormone-acid Solution in an Atmosphere of Nitrogen Gas and in an Atmosphere of CO_2 Gas

In one of these experiments the hormone was hydrolyzed with 20% H_2SO_4 and in another experiment with 10% H_2SO_4.

60 mg. of hormone were added to 1 cc. of 20% H_2SO_4. A stream of nitrogen gas was passed into the tube above the acid-hormone solution until all atmospheric oxygen was displaced as indicated by a negative test with manganous hydroxide solution. The tube containing the hormone and acid was placed in a boiling water bath for ½ hour. At the end of the heating period the contents of the tube were immediately cooled by immersing the tube in a mixture of ice and water. When cooled, the stream of nitrogen gas was removed. Potency tests were then carried out.

Results:

Dog	Sex	Wt. kg.	Dose	Init. Ca.	Final Ca.	Ca. Increment
D1	M	21	60 mg.	11.54	11.64	0.10
				11.54	11.54	0.00

A second experiment of this type was carried out as follows:

55.69 mg. of hormone were placed in a flask and 2 cc. of 10% H_2SO_4 were added. The flask was then stoppered with a two-hole stopper. A reflux condenser was fitted into one hole and a glass tube connected to a nitrogen tank was inserted into the other hole. The upper stem of the condenser was attached through a gas trap to a water trap.

Nitrogen gas was passed through the flask until all the air was displaced. After the gas escaping from the exit tube was oxygen-free, the hot water bath was heated to boiling and kept at that temperature for 1 hour. The flask was then cooled by placing a towel around the flask and immersing it in a cold water bath. The contents of the flask were removed, neutralized with $NaHCO_3$, made up to a volume of 10 cc., and tested for potency.

Results:

Dog	Sex	Wt. kg.	Dose	Init. Ca.	Final Ca.	Ca. Increment
6	F	15.91	55.69 mg.	11.47	12.09	0.62
				11.47	12.04	0.57

It is seen from the above results that the potency of the hormone is greatly decreased in this case also.

An experiment similar in nature was carried out using CO_2 to displace and exclude atmospheric oxygen. The following procedure was used:

$2\frac{1}{2}$ cc. of distilled water were mixed with 75 mg. of hormone. This was diluted with $2\frac{1}{2}$ cc. of 10% H_2SO_4 making the final concentration of 5% H_2SO_4 solution. The hormone-acid solution was boiled for 1 hour in a flask in a stream of CO_2. At the end of the heating period the contents of the flask were removed, neutralized, and tested for potency.

Results:

Dog	Sex	Wt. kg.	Dose	Init. Ca.	Final Ca.	Ca. Increment
J-1	M	16.36	58.26 mg.	10.32	12.04	1.72

The last experiment indicates that the hormone molecule is comparatively stable to boiling dilute H_2SO_4.

(4) Hydrolysis of the Hormone in an Acid Solution Under Increased Pressure and Temperature

A series of hydrolysis experiments were carried out in which the hormone hydrolyzed under increased pressure and temperature in different concentrations of acid for varying lengths of time.

From table 4 it is seen that increase of free amino

acid nitrogen and destruction of potency of the hormone are accelerated by heating in an acid solution under increased pressure and temperature.

It would appear from the following experiments that the hormone is destroyed by heating under increased pressure, and that added acid is not necessary.

When the hormone preparation is heated in distilled water for 1 hour under increased pressure and temperature the potency is not affected, but when it is heated in distilled water for 4 hours under the same conditions the potency is completely destroyed (Table 4).

When the hormone is heated in buffer pH of 7.0 for two hours under increased pressure and temperature the potency is reduced to one-half of its original strength, but when it is heated in buffer pH of 7.0 for 5 hours under the same conditions there is no potency left in the preparation- (Table 4).

ADSORPTION STUDIES

A number of experiments were performed using Lloyd's reagent and activated charcoal as the adsorbing agents. The hormone could be adsorbed on charcoal and Lloyd's reagent in alkaline solution but could not be released as shown by potency tests and by measurements of total nitrogen by

TABLE IV

HORMONE HEATED UNDER INCREASED PRESSURE AND TEMPERATURE (15-20 LBS., 250°--259° F.)

Concentration	Length of Heating	Ca Increment mg. %	% of Amino N of Total N.
10% HCl	45 min.	0.53 mgs.	---
0.1 N. HCl	1 hr.	0.65	13.81%
0.05 N. HCl	2 hrs.	0.18	11.04%
		*2.03)	
		*2.14)	
0.033 N. HCl	3 hrs.	0.00)	10.71%
		0.06)	
Distilled water	1 hr.	5.96)	---
		5.85)	
		6.65	
Distilled water	4 hrs.	0.00	9.64%
Buffer pH 7.0	2 hrs.	2.58	---
Buffer pH 7.0	5 hrs.	0.00	---

* Dog received dose and half of hydrolyzed hormone per kilo of body weight.

the Koch-McMeekin micro kjeldahl method. It would appear, then, that the hormone is protein in nature.

The hormone could not be adsorbed on bone ash.

THE EFFECT OF REDUCTION OF THE HORMONE BY SODIUM IN LIQUID AMMONIA

This phase of the investigations in which I have participated has been published in full in Vol. 112, 1935, of the Journal of Biological Chemistry under the following title, "Some Reactions of Ammonolyzed Parathyroid Hormone", by Richard G. Roberts, Wilbur R. Tweedy, and George H. Smullen.

PART II

STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID HORMONE

ATTEMPTS TO SHOW ABSORPTION FROM THE G. I. TRACT

Four lines of endeavor were followed in order to determine whether parathyroid hormone is absorbed from the G. I. tract to produce an increase in blood serum calcium.

1. Administration of Hormone Via Stomach Tube

In the first method used a double standard dose of hormone (7 mg. per kilogram of body weight) was dissolved in a saturated solution of $NaHCO_3$ and administered to a dog via stomach tube. Blood samples were taken at various intervals and the serum analyzed for calcium.

All variations in the calcium values were found to be within the limits of physiological variations.

(1) Administration of hormone in a saturated solution of $NaHCO_3$.

Dog	Sex	Wt. kg.	Dose	Blood Serum Ca.	Time	Ca. Increment
N5	M	14	98 mgs.	10.89	Initial	----
				10.89	1 hr, 15'	0.00
				11.12	17 hrs,30'	0.23
			73.5 mgs.	-----	19 hrs.	----
				11.24	24 hrs,30'	0.35

In another experiment of this type the hormone preparation was dissolved in a neutral solution (water) and was administered via stomach tube. The blood-serum-calcium values all fell within the range of physiological variation.

(2) Administration of hormone in neutral solution.

Dog	Sex	Wt. kg.	Dose	Time	Blood Serum Ca.	Ca. Increment
N6	M	10.7	74.9	0	10.61	----
			----	5 hrs.	10.78	0.17
			74.9	5 hrs. 30'	-----	----
				26 hrs.	11.04	0.43

A third experiment of this nature was performed by mixing the hormone preparation with a 4% solution of calcium gluconate. The hormone-calcium gluconate solution was made just alkaline to phenolphthalein and administered to a dog through a stomach tube.

The hormone was mixed with calcium gluconate in the hope that calcium gluconate would raise the blood serum calcium and that if the hormone were absorbed as such it would maintain the blood serum calcium at a high level for a long period of time. Apparently the hormone had no effect when administered in the above manner.

(3) Administration of hormone in 4% calcium gluconate solution.

Dog	Sex	Wt. kg.	Dose	Time	Blood Serum Ca.	Ca. Increment
N7	M	8.18	57.26 mg.	0	11.01	----
				-	11.01	0.00

In a fourth experiment of this nature the hormone was brought into an emulsion with oil with the hope that the oil would protect the hormone particles from the action of acid in the stomach until the mixture reached the intestine where it would be absorbed. This experiment illustrates also that the hormone does not increase the blood serum calcium when administered orally in the manner described.

(4) Administration of hormone in olive oil suspension.

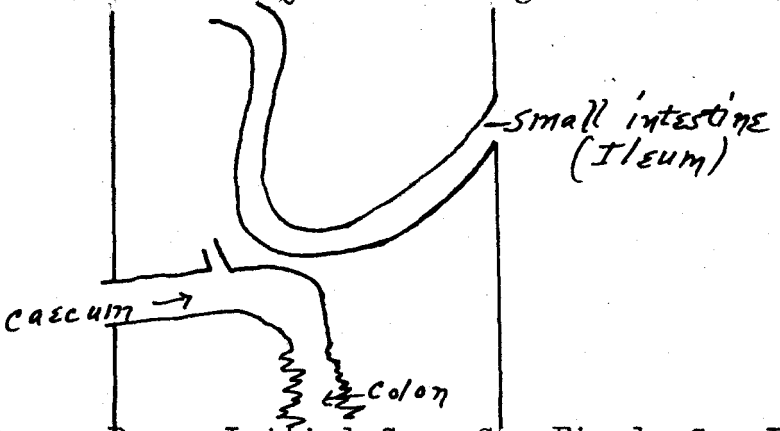
Dog	Sex	Wt. kg.	Dose	Time	Blood Serum Ca.	Ca. Increment
N8	M	13.7	95.9 mg.	0	10.67	----
				18 hrs.	10.67	0.00

2. Administration of Hormone Via Iliostomy

The second method employed to attempt to demonstrate whether the hormone was absorbed as such from the G. I. tract and produced an increase in blood serum calcium wasperformed by placing hormone into the intestine of a dog having an iliostomy.* An iliostomy was made about 3 years ago on this

*This experiment was carried out at the University of Chicago in collaboration with Dr. R. D. Templeton of the Department of Physiology of Loyola University School of Medicine.

dog. In this preparation the intestinal contents come to the outside on the abdominal wall. None of the contents of the small intestine reach the large intestine. In addition to an iliostomy this animal had a cecostomy (appendictostomy). The hormone material was injected through the cecum into the upper colon.



Dog	Sex	Wt. kg.	Dose	Initial Ca.	Ca. Final	Ca. Increment
X	F	20	105 mg.	8.14	8.14	0.00

This method also failed to show any increase in blood serum calcium.

3. Administration of Hormone Via Thiry Fistula

The third method used to determine whether the hormone would raise the blood serum calcium when given via the G. I. tract was performed by placing the hormone into a Thiry's (12) fistula of a dog. In one experiment the hormone was administered in a physiological saline solution and in another experiment the hormone was mixed with blood serum of the same dog since blood serum is absorbed by selective absorption. Blood samples were then taken before the hormone

preparation was placed into Thiry's fistula and the second samples were taken 15 hours and 17 hours later respectively and analyzed for blood serum calcium.

(a) Absorption of hormone-saline solution in Thiry fistula

Dog	Sex	Wt. kg.	Dose	Init. Ca.	Final Ca.	Ca. Incr't.
Thiry Fistula	M	15.4	107.8 mg.	11.10	11.10	0.00

(b) Absorption of hormone-blood serum in Thiry fistula.

Dog	Sex	Wt. kg.	Dose	Init. Ca.	Final Ca.	Ca. Incr't.
Thiry Fistula	M	15.4	110 mg.	11.10	11.10	0.00

4. Hormone-feeding in Food Mixtures to Rats

Feeding parathyroid hormone to rats has a decided advantage over feeding the hormone to dogs via stomach tube because the hormone is administered almost continuously in infinitely small doses to rats due to their feeding habits.

a) Normal rats on adequate diet

Four male rats were placed on a fox-chow diet,* two of which received a total of 200 units of parathyroid hormone mixed with 15 grams of fox-chow food per day for four days. Tap water was supplied freely. At the end of the four day

*Fox chow diet manufactured by Purina Mills.

period blood samples were taken by cardiac puncture. The rats were killed, decapitated, and the heads sent to Dr. I. Schour, Department of Dental Histology, University of Illinois, for examination of the teeth.

The experimental rats gave blood serum calcium values of 11.10 mg. % and 11.24 mg. % while the control rats gave blood serum calcium values of 10.32 mg. % and 10.32 mg. %. The experimental rats showed a slight increase in the blood serum calcium over the control rats.

b) Thyroparathyroidectomized rats on an adequate diet.

The results of the above experiment led us to determine whether thyroparathyroidectomized rats could utilize parathyroid hormone when mixed with an adequate diet. In these cases there is a definite need for parathyroid hormone.

Four rats were thyroparathyroidectomized three days preceeding the beginning of the experiment. Each rat was placed in a separate cage;--two control rats and two experimental rats. Tap water was provided for drinking purposes.

15 grams of fox-chow rat food were placed in each control cage. 15 grams of fox-chow rat food were mixed thoroughly with 100 units of parathyroid hormone preparation for each experimental rat. These food mixtures were fed for three days. When this feeding period was terminated, blood samples were

taken by cardiac puncture and blood serum calcium values determined.

The control rats showed a serum calcium value of 6.45 mg.% and 6.75 mg.%. The experimental rats gave serum calcium values of 8.04 mg.% and 7.64 mg.%. The average control calcium value was 6.60 mg.%, while the average experimental rat serum calcium value was 7,84 mg.%. The apparent average blood serum calcium increment was 1.24 mg.%. The above values may be due to individual variation, but both experimental rats show higher serum calcium values than that of the control rats.

c) Thyroparathyroidectomized and parathyroidectomized rats placed on a salt-free diet*

Four experiments of this nature were carried out. In each case a group of control parathyroid rats were fed the salt-free diet without any parathyroid hormone added to it. In one experiment blood serum calcium values were used to determine whether the rats were able to assimilate the hormone as such and produce an increase in serum calcium. In another experiment absence of tetany was used as an index of hormone assimilation. A rat was considered in tetany when after spinning it

*Salt free diet consists of : casein 18 gms., starch 50 gms., butter 15 gms., yeast 15 gms., cod liver oil 2 gms.

by its tail and setting it down it would drag one of its hind legs.

In the other two experiments the rats were placed in an incubator set at 91-92°F. and prevention of death was taken as an index of hormone assimilation. In one of these experiments the rats were kept on a salt-free diet thirteen days previous to operation.

All of these experiments tend to indicate that the parathyroid hormone when fed in a salt-free diet to parathyroidectomized rats does not raise the blood serum calcium, does not prevent death, but may exert a slight tendency in preventing tetany. However, due to the possible presence of accessory parathyroids, the series of experiments performed does not present conclusive evidence.

EXPERIMENT I

Parathyroidectomized and Thyroparathyroidectomized Rats Placed on a Salt-free Diet with Parathyroid Hormone

5 experimental rats fed 25 gms. of salt-free diet mixed with 400 units of parathyroid hormone. 5 control rats fed 25 gms. of salt-free diet. Tap water supplied freely to both groups.

X12, x13, x14, x15--29 days old. All the rest of the

rats were 27 days old.

Length of experiment--2 days

Control Rats		Experimental Rats	
Serum Calcium Value		Serum Calcium Value	
(1)	6.80	(1)	6.50
(2)	8.00	(2)	8.20
(3)	5.80	(3)	6.00
		(4)	4.80

(2 Control rats died.)

EXPERIMENT II

Thyroparathyroidectomized Rats Placed on Salt-free Diet

Tetany used as index of hormone deficiency.

Rats--24 days old. 4 experimental rats fed 10 gms. of salt-free diet mixed with 500 mgms. of hormone twice for two days.

4 control rats fed 10 gms. of salt-free diet twice for 2 days.

Tap water supplied freely to both groups. Temperature ranged from 19°C. to 22°C. Length of experiment--3 days.

First Reading--23 Hours After Operation

Controls	Experimentals
0------T	RC1----O.K.
1R---O.K.	RC------O.K.
RC3----T	R3------O.K.
R2-----T	RC2-----O.K.

Second Reading--28.5 Hours After Operation

Controls	Experimentals
2R-----T	RC------O.K.
RC3----T	R3--------T
00-----T	RC2-----O.K.
R1-----T	RC1-----O.K.

Third Reading--41 Hours After Operation

Controls	Experimentals
RC3----T	R3------O.K.
2R---O.K.	RC------O.K.
R1---O.K.	RC1-------T
0------T	RC2-----O.K.

Fourth Reading--43 Hours After Operation

Controls	Experimentals
RC3----T	R3------O.K.
R1-----T	RC1-------T
00-----T	RC------O.K.
R2-----T	RC2-------T

Fifth Reading--45 Hours After Operation

Controls	Experimentals
RC3----T	R3-----O.K.
R1-----T	RC1------T
00-----T	RC-----O.K.
R2----- T	RC2------T

EXPERIMENT III

Thyroparathyroidectomized Rats Placed on a Salt-free Diet and Parathyroid Hormone Placed in Incubator Set at 91-93°F., Using Prevention of Death as Index of Assimilation of Hormone.

8 Thyroparathyroidectomized rats:

4 control rats fed 10 gms. of salt-free diet each day for 2 days.

4 experimental rats fed 10 gms. of salt-free diet plus 500 mgs. of hormone each day for 2 days.

Tap water freely supplied.

Rats 24 days old, and of the same litter.

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1 Control rat dead 16 hours after operation.

2 Experimental rats dead 22 hours after operation.

1 Experimental rat dead 40 hours after operation.

At the end of the experiment 3 control rats were living while only 1 experimental rat was alive.

EXPERIMENT IV

Rats Placed on Salt-free Diet for 13 Days Preceding Thyroparathyroidectomy Fed Hormone Plus Salt-free Diet

10 rats placed on salt-free diet for 13 days previous to thyroparathyroidectomy. 5 experimental rats fed 25 gms. of salt-free diet plus 1,000 mgs. of hormone. 5 control rats fed 25 gms. of salt-free diet. Both groups received tap water freely, and were placed in an incubator set at 91-92°F.

17.5 Hours After Operation

Controls	Experimentals
1 Dead	4 Dead
2 Tetany	1 Living O.K.
2 Living O.K.	

41.5 Hours After Operation

Controls	Experimentals
1 Dead	4 Dead
3 Tetany	1 Tetany
1 Living O.K.	

INTRAVENOUS ADMINISTRATION OF PARATHYROID HORMONE

Allardayce (13) found that the maximum increment of the blood serum calcium induced with "Parathormone", administered

intravenously, occurred four to eight hours, rather than 15 to 24 hours as has been established for intramuscular and subcutaneous injections. The response obtained from intravenous injection was considerably less.

Intravenous injections of our hormone preparations were made, using a standard dose, double standard dose, standard dose immediately followed by sub-standard doses at regular intervals, and double standard dose followed by sub-standard doses at regular intervals.

In many cases the same dogs were given similar doses of parathyroid hormone subcutaneously at a later date, and the blood serum calcium values compared with those given intravenously. It appears from the results that a double standard dose of hormone administered intravenously gives no greater serum calcium value than a regular standard dose injected intravenously.

The maximum serum calcium increment, when the hormone is injected intravenously, occurs 4 hours after administration. The serum calcium increment (1.25 mg.) produced by standard or double-standard dose of hormone injected intravenously is only $\frac{1}{4}$ of the effect of standard dose of hormone injected subcutaneously.

The accompanying tables show the results obtained from intravenous injections using different dosages of the hormone preparation.

Double-standard Dose Injected Intravenously

Dog	Sex	Wt. kg.	Dose	Time	Serum Ca. Value	Ca. Increm't
0-1	M	11	77	0	10.67	----
				2 hrs. 5'	11.81	1.14
				4 hrs. 5'	11.92	1.25
				6 hrs. 50'	11.70	1.03
				23 hrs. 5'	10.55	0.12

Subcutaneous Injection

Dog	Sex	Wt. kg.	Dose	Initial Ca.	Final Ca.	Ca. Increment
0-1	M	11	38.5	10.53	13.76	3.23

Standard Dose Injected Intravenously

Dog	Sex	Wt. kg.	Dose	Time	Serum Ca. Value	Ca. Incr't
0-2	M	26.8	95 mg.	0	10.89	----
				4 hrs.	12.16	1.27
				6 hrs. 45'	11.70	0.81
				8 hrs.	11.58	0.69
				23 hrs. 45'	11.24	0.35

Injected Subcutaneously

Dog	Sex	Wt. kg.	Dose	Initial Ca.	Final Ca.	Ca. Increment
0-2	M	26.8	94	10.84	10.85	4.01

Double-standard dose of hormone injected intravenously in sub-dosages compared to a standard dose injected subcutaneously in sub-dosages.

Intravenous Injections

Dog	Sex	Wt. kg.	Dose	Time	Ca. Value	Ca. Increment
0-3	M	17.3	25 mg.	0	11.81	----
			25 mg.	30'	11.81	0.00
			25 mg.	1 hr.	11.70	-0.11
			25 mg.	1 hr. 45'	11.47	-0.34
			------	3 hrs.45'	11.93	+0.12
			-----	5 hrs.45'	11.93	+0.12
			-----	21 hrs.45'	11.47	-0.34

Subcutaneous Injections

Dog	Sex	Wt. kg.	Dose	Initial Ca.	Final Ca.	Ca. Increment
0-3	M	17.3	60.55 mg. in ¼ sub-dosages 4 x, ½ hr. apart each time.	11.47	14.45	2.98

Double standard dose of hormone injected intravenously followed by sub-doses of hormone injected intravenously.

Dog	Sex	Wt. kg.	Dose	Time	Ca. Value	Increment
0-4	M	28.63	200.4	0	10.43	----
			25	3 hrs.	11.18	0.75
			25	3 hrs. 45'	-----	----

(continued)

Dog	Sex	Wt. kg.	Dose	Time	Ca. Value	Increment
0-4	M	28.63	23	4 hrs. 15'	-----	----
			--	5 hrs. 45'	11.87	1.44
			--	21 hrs.	12.73	2.30

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